iGEM 2016: Pittsburgh
Week 13 Lab Notebook

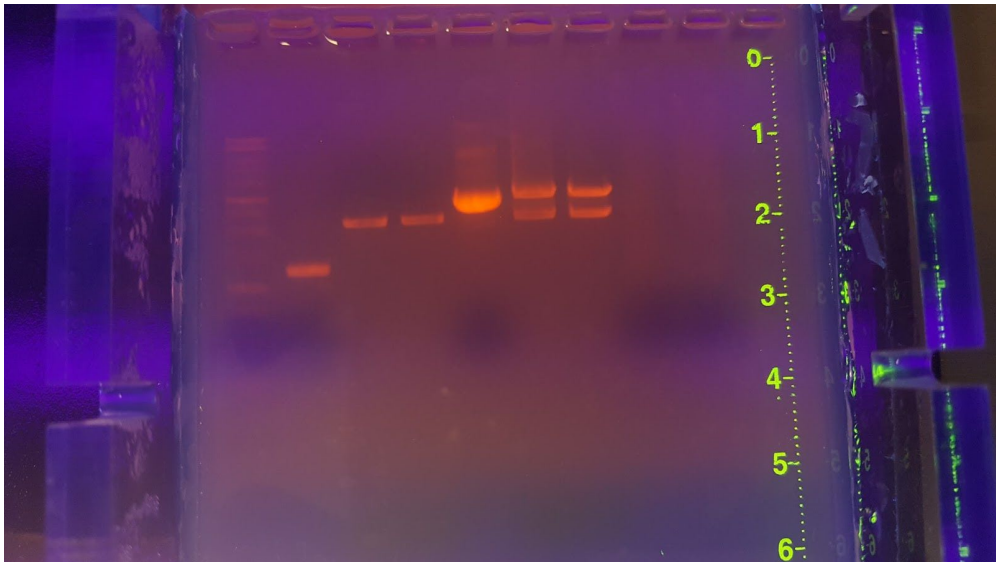
Monday, August 15

LacZ Site Directed Mutagenesis (Maya)

Protocol

Transformed on Kanamycin plates

Miniprepped and gel checked PT3-T3 and PT7-T3 (Maya)



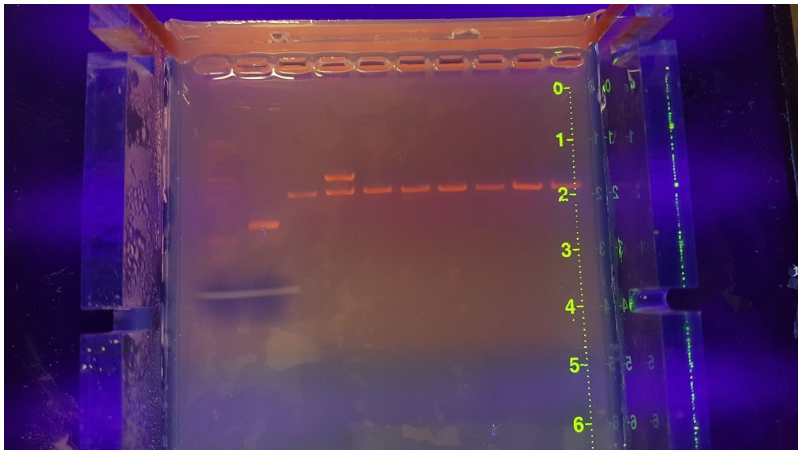
Lane 1: 1kb ladder
Lane 2: PT7 control
Lane 3: PT7-T3 3:1
Lane 4: PT7-T3 7:1
Lane 5: PT3 control
Lane 6: PT3-T3 3:1
Lane 7: PT3-T3 7:1
Lane 8: LacZ Mut. pair 1
Lane 9: LacZ Mut. pair 2

Liquid cultures of PT3-RBS-Backbone and PT7-RBS-T3 (Maya)

Tuesday, August 16

Miniprep liquid cultures of PT3-RBS-Backbone and PT7-RBS-T3 (Maya)

Gel check PT7-T3 (10 ul rxn, 1ul DNA) (Maya)



Lane 1: 1kb ladder
Lane 2: PT7-RBS control
Lane 3: Colony 1
Lane 4: Colony 2
Lane 5: Colony 3
Lane 6: Colony 4
Lane 7: Colony 5
Lane 8: Colony 6
Lane 9: Colony 7
Lane 10: Colony 10

Colony 2 successful ligation

No growth on LacZ plates (Maya)

Wednesday, August 17

Weekly Meeting

Transform plasmids for InterLab (Claire)

5 uL device into 50 uL competent cells

iGEM protocol

Make 1 L (500 mL, 250 mL x 2) LB broth (Claire)

Gel extraction of PT3-RBS-T3 and PT7-RBS-T3 (Maya)

Digest: 40 uL rxn (2 ul EcoRI, 2 ul SpeI, 4 ul Buffer)

5 ug DNA

PT7-T3

12.15 uL DNA

19.85 uL H2O

PT3-T3 1

12.34 uL DNA

19.66 uL H2O

PT3-T3 2

13.54 uL DNA

18.46 uL H2O

Extended incubation (45 min 37, 20 min 65)

Lane 1: 1kb ladder
Lane 2: PT7-T3 uncut
Lane 3: PT7-T3 cut
Lane 4: PT7-T3 cut
Lane 6: PT3-T3 1 cut
Lane 7: PT3-T3 1 cut
Lane 8: PT3-T3 2 cut
Lane 9: PT3-T3 3 cut

Lane 5: PT3-T3 uncut

Linearize Terminator (Maya)

1 ug, 20 uL Rxn

1 ul EcoRI
1 ul XbaI
2 ul Buffer
5.50 ul DNA
10.5 ul H2O

Set up 3 sequencing reactions for PT3-RBS-Backbone, did not get PO in time to send out (Maya)

Plated PT3-GFP bacterial stab from AddGene on Amp plates (Maya)

Thursday, August 18

Ligation of PT3-RBS-T3 PT7-RBS-T3 to terminator (Maya)

1 uL T4 Ligase
2 uL T4 Ligase Buffer
PT3-RBS-T3

Control:	1 ul Vector
	0 ug Insert
	16 ul H2O
3:1	1 ul Vector
	3.857 ug Insert
	--- ul H2O
7:1	1 ul Vector
	9.00 ug Insert
	--- ul H2O

PT7-RBS-T3

Control:	1 ul Vector
	0 ug Insert
	--- ul H2O
3:1	1 ul Vector
	3.857 ug Insert
	--- ul H2O
7:1	1 ul Vector
	9.00 ug Insert
	--- ul H2O

Transform with Cheryl's cells (Maya)

LacZ Mutagenesis with DMSO and Cheryl's cells (if left over from ligation) (Maya)

Pack/Clean lab for move to Alex's lab?? (Maya/Claire)

Liquid cultures of InterLab devices, 2 cultures per device (Claire)

5 devices, 2 cultures each
2 tubes of LB + chloramphenicol

Transform plasmids A-H from William and Mary (Claire)

Lids snapped off F, G, and H in centrifuge before ice incubation
Moved cells to new tubes, fairly certain correctly labeled

Cleavage reaction with lead DNAzyme G (Claire)

50 uL reaction: 5 uL 50 nM : 50 uM annealed duplexes
4.18 uL lead at 24 uM (2 uM final concentration)
40.8 uL water

24 uM lead = 1 part 96.5 uM lead : 3.02 parts water
5:15.1

Incubate at 37 and RT for 1 hour
Quench on ice

Cell-free reaction with lead DNAzyme (Claire)

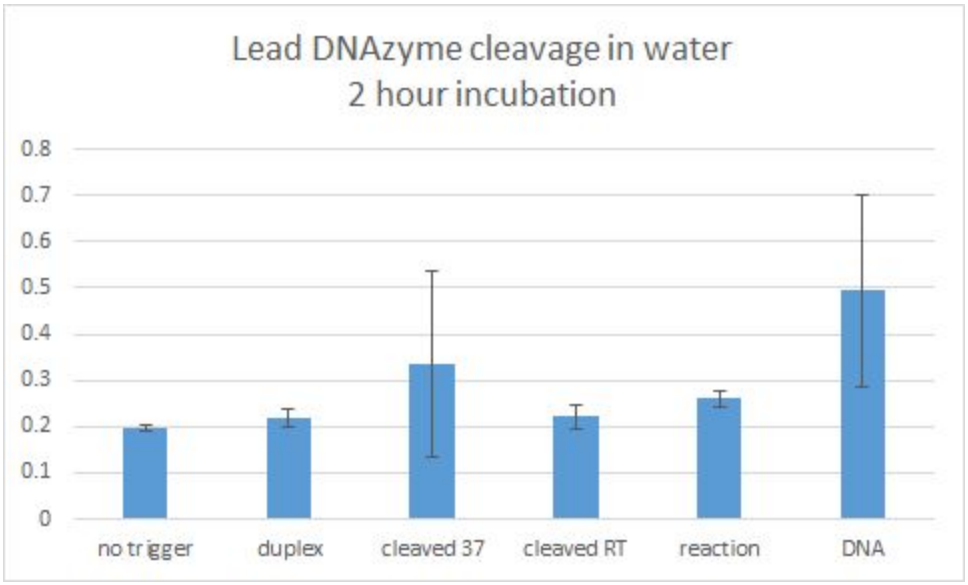
	No trigger M1-2	Duplex M3-4	37 reaction M5-6	RT rxn M7-8	Rxn parts M9-10	DNA trig. M11-12
Solution A	2	2	2	2	2	2
Solution B	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	.25	.25	.25	.25	.25	.25
substrate	.25	.25	.25	.25	.25	.25
Switch (25 ng)	.30	.30	.30	.30	.30	.30
trigger	.70 water	.70 5 nM : 5 uM (Pb G 1000)	.70 5 nM : 5 uM, 2 uM lead, 1 hr incubation	.70 5 nM : 5 uM, 2 uM lead, 1 hr incubation	.07 uL 50 nM : 50 uM (Pb G) 0.63 15.87 uM lead (2 uM)	.70 5 nM DNA oligo trigger

15.87 uM lead = 1 part 96.5 uM lead : 5.08 parts water

12 reactions, make 14:
Solution A: 28 uL
Solution B: 21 uL
RNAse inhibitor: 3.5 uL
Substrate: 3.5 uL
Switch at 84.1 ng/uL: 4.2 uL

Aliquot into 6, each 8.6 uL
Add appropriate triggers
Add 1.4 uL DNA trigger at 25 nM to leftover master mix (M13)
Absorbance at 2 hr = 2.1025, overnight = 3.5369

Condition	1	2	average	st dev
no trigger	0.2017	0.1935	0.1976	0.005798
duplex	0.2322	0.2056	0.2189	0.018809
cleaved 37	0.4791	0.1939	0.3365	0.201667
cleaved RT	0.2035	0.2393	0.2214	0.025314
reaction	0.2471	0.2735	0.2603	0.018668
DNA	0.6416	0.3459	0.49375	0.209091



View from plate bottom

Not much activation from completed reactions--use lead buffer tomorrow

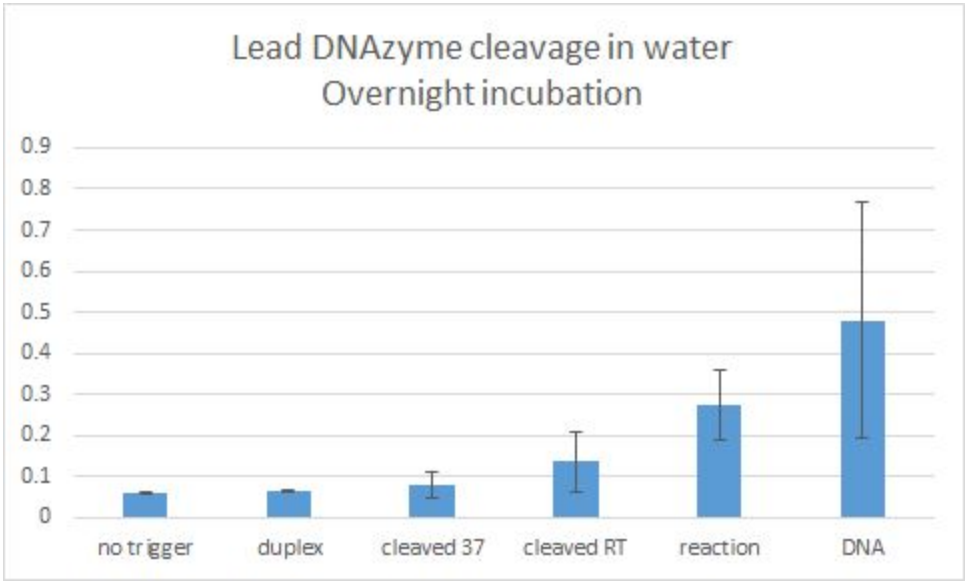
Place in incubator after reading

Friday, August 19

Yesterday's plate (lead DNAzyme cleavage cell-free test) darkened in color

Condition	1	2	average	st dev
no trigger	0.0627	0.0599	0.0613	0.00198
duplex	0.0653	0.0613	0.0633	0.002828

cleaved 37	0.103	0.0588	0.0809	0.031254
cleaved RT	0.0854	0.1871	0.13625	0.071913
reaction	0.2148	0.3358	0.2753	0.08556
DNA	0.2789	0.6827	0.4808	0.28553



Reaction condition seems to give best results, but large variation

InterLab cell growth and assay (Claire)

Prepare 12 15-mL Falcon tubes of 10 mL LB with CM

Remove 100 uL from overnight cultures, plate Row H.

Read OD_{600}:

3	sample (replicate 1)	Abs600 reading	volume of preloading culture	volume of preloading media
4	positive control	0.48	0.462962963	9.53703704
5	negative control	0.354	0.653594771	9.34640523
6	device 1	0.397	0.573065903	9.4269341
7	device 2	0.371	0.619195046	9.38080495
8	device 3	0.384	0.595238095	9.4047619
9	media+chl	0.048	#DIV/0!	#DIV/0!
10				

sample (replicate 2)	Abs600 reading	volume of preloading culture	volume of preloading media
positive control	0.437	0.514138817	9.48586118
negative control	0.368	0.625	9.375
device 1	0.243	1.025641026	8.97435897
device 2	0.393	0.579710145	9.42028986
device 3	0.368	0.625	9.375
media+chl	0.048	#DIV/0!	#DIV/0!

Dilute cultures to 0.02: remove appropriate amount of prepared LB and replace with culture. Round numbers to the ones place (used P1000)

Samples added directly to plate, plate kept on ice

Plate rinsed with DI water from FITC and LUDOX protocols

Condensation in wells by hour 4

Last sample cut short by about 20 min to ensure access to plate reader

Liquid cultures of William and Mary plasmids (Claire)

2 cultures per device (A1, A2, B1, B2,...,H1, H2)

Fold lead hairpin DNAzyme (Claire)

3 conditions

Resuspended only (original/as is), 74 uM

Denaturing conditions (95 degC for 3 min, cool to room temp), 74 uM

Annealing protocol: 1 ug oligo = 66.9 nM — 1.79 uL 18.7uM resuspended DNA, 5 uL 10X T4 DNA ligase buffer, 43.21 uL water

Lead solution: $Pb(CH_3CO_2)_2 \cdot 3H_2O$ from Sigma-Aldrich, 379.33 g/mol

100 mM = 379.33e-3 g / 1 mL water

Cleavage reaction with lead DNAzyme G (Claire)

1:1000 duplex — 5 uL 50 nM : 50 uM annealed duplex, 4.18 uL $Pb(CH_3CO_2)_2 \cdot 3H_2O$ at 24 uM, 40.8 uL Pb buffer pH 7.26

Hairpin — 5 uL 37.4 nM folded hairpin (3 separate conditions), 4.18 uL $Pb(CH_3CO_2)_2 \cdot 3H_2O$ at 24 uM, 40.8 uL Pb buffer pH 7.26

Incubate at 37 degC or room temp for 1 hour

Quench on ice

*low on annealed duplex for RT condition

Anneal more G lead DNAzyme duplex 50 nM : 50 uM, 25 uL reaction (Claire)

1.25 uL 1 uM substrate

13.89 uL 90 uM catalytic

2.5 uL T4 DNA ligase buffer

7.36 uL water

Cell-free reaction with lead DNAzyme (Claire)

25 ng switch

3.74 nM trigger (didn't spec resuspended hairpin until after cleavage was set up)

Condition	Trigger (0.70 uL unless otherwise stated)
No trigger	water
duplex	3.74 nM : 3.74 uM annealed
Hairpin original	3.74 nM resuspended hairpin
Hairpin denature	3.74 nM hairpin heated to 95 degC
Hairpin anneal	3.74 nM hairpin heated to 85 degC with T4 ligase buffer
Duplex 37	3.74 nM : 3.74 uM duplex that underwent cleavage reaction at 37 deg incubation
Duplex RT	3.74 nM : 3.74 uM duplex that underwent cleavage reaction at room temp incubation
Denatured 37	3.74 nM hairpin heated to 95 that underwent cleavage reaction at 37 deg incubation
Denatured RT	3.74 nM hairpin heated to 95 that underwent cleavage reaction at room temp incubation
Duplex rxn	0.07 uL 37.4 nM : 37.4 uM annealed duplex 0.417 uL 24 uM lead solution 0.213 uL Pb buffer pH 7.26
Denatured rxn	0.28 uL 66.9 nM hairpin heated to 95 deg 0.417 uL 24 uM lead solution 0.003 uL Pb buffer pH 7.26
DNA	3.74 nM oligo

3.74 nM concentration= 1 part 5 nM plus 0.34 parts water

12 conditions, duplicate, make 26:

- Solution A: 52 uL
- Solution B: 39 uL
- RNAse inhibitor: 6.5 uL
- Substrate: 6.5 uL
- Switch at 84.1 ng/uL: 7.8 uL

Add 1.4 uL appropriate triggers

Aliquot master mix into 12, each 8.6 uL

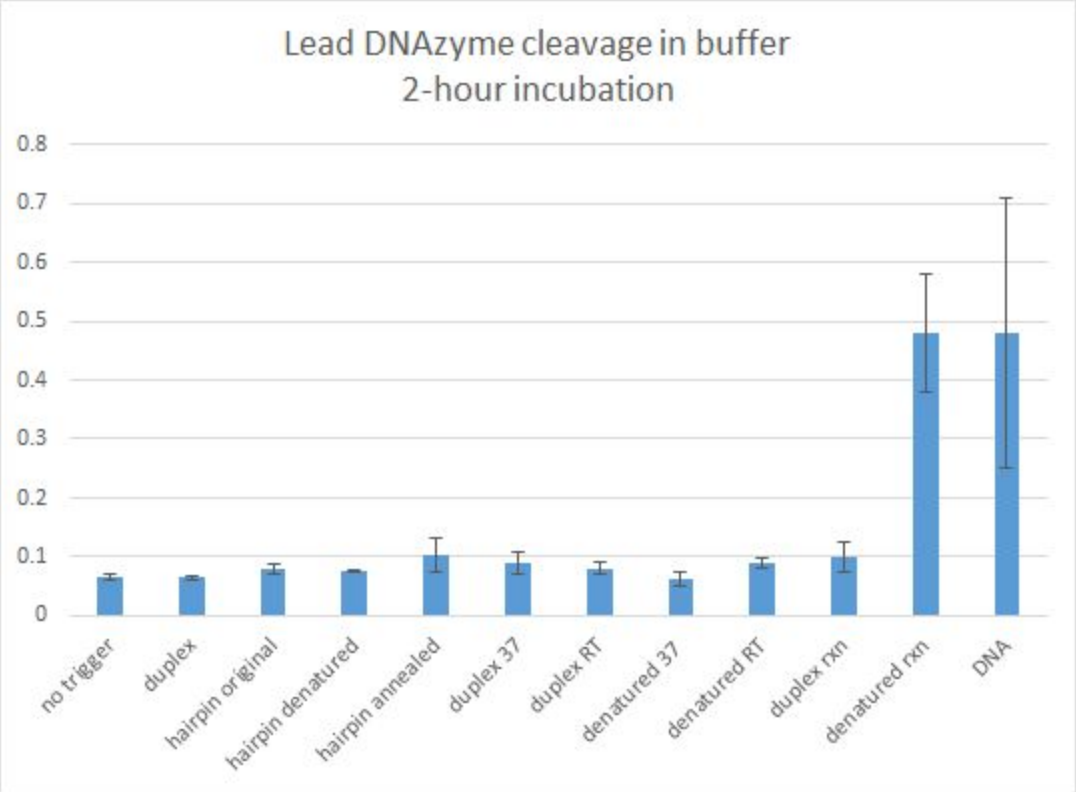
Split into 24 tubes, each 5 uL volume

Incubate in heat block for about 1.5 hours (to ensure access to plate reader)

Read absorbance at 570 nm:

Condition	1	2	average	st dev

no trigger (N1-2)	0.0613	0.0683	0.0648	0.00495
Duplex (N3-4)	0.0623	0.067	0.06465	0.003323
hairpin original (N5-6)	0.0744	0.086	0.0802	0.008202
hairpin denatured (N7-8)	0.0759	0.0748	0.07535	0.000778
hairpin annealed (N9-10)	0.1228	0.083	0.1029	0.028143
duplex 37 (N11-12)	0.1018	0.0763	0.08905	0.018031
duplex RT (N13-14)	0.0882	0.0726	0.0804	0.011031
denatured 37 (N15-16)	0.0708	0.0545	0.06265	0.011526
denatured RT (N17-18)	0.0968	0.0841	0.09045	0.00898
duplex rxn (N19-20)	0.1176	0.0826	0.1001	0.024749
denatured rxn (N21-22)	0.5504	0.4094	0.4799	0.099702
DNA (N23-24)	0.6407	0.3164	0.47855	0.229315



View from plate bottom

Place in incubator

Hairpin looks much more promising than duplex. Rxn better than cleaved

Miniprep PT3-GFP (Maya)

Concentrations: 75.9, 95.5, 83.0, and 101.7 ng/ul

No growth on PT3-T3-Term, PT7-T3-Term, or LacZ Mutagenesis Plates (Maya)

Sent out PT3-RBS out for sequencing (Maya)

Resuspended PT7-GToehold-sense/antisense (Maya)

40uM

Saturday, August 20

Remove plate from incubator

Cleavage reactions darkened, but so did hairpins

Read next week

Miniprep William and Mary plasmids (Claire)

Concentrations (ng/uL):

A1: 258.9	E1: 262.9
A2: 195.2	E2: 276.0
B1: 297.0	F1: 228.9
B2: 343.0	F2: 266.0
C1: 304.1	G1: 257.1
C2: 302.3	G2: 227.6
D1: 283.9	H1: 260.5
D2: 255.3	H2: 262.5